

Reactions of Fatty Materials with Oxygen. XVI.¹ Relation of Hydroperoxide and Chemical Peroxide Content to Total Oxygen Absorbed in Autoxidation of Methyl Oleate²

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MOST workers in this field are now in agreement that α -methylene attack to form hydroperoxides is the major reaction in the autoxidation of monoethenoic and non-conjugated polyethenoic fatty esters. To account satisfactorily for the energetics of the reactions involved however, several investigators have proposed that in the autoxidation of monoethenoic compounds, the initial point of oxidative attack is not at the α -methylene position but at the double bond. Only a slight amount of double bond attack is required to "trigger" the predominating α -methylene chain reaction (1, 2, 2a, 3).

An opportunity to obtain direct experimental verification for the conclusion that hydroperoxides are the predominant initial products of autoxidation recently became available with the development of the polarographic method for determining hydroperoxides in autoxidation mixtures (12, 13). The correlation of results obtained by this method with those obtained by the chemical or iodometric method of analysis makes it possible to learn what proportion of the total peroxide content is made up of hydroperoxide, and whether in some stage of the reaction all the peroxide is present as hydroperoxide.

In a study of this type it is also essential that oxygen absorption be followed quantitatively since it is not only important to know the ratio of hydroperoxide to total peroxide but also the ratio of total peroxide to oxygen absorbed. Relatively few investigators have examined the quantitative aspects of oxygen absorption of methyl oleate (4, 8).

The use of manometric techniques offers a convenient method for measuring the amount as well as the rate of absorption of oxygen. Much of the earlier quantitative work on the autoxidation of olefins has been carried out by measuring the change in volume of oxygen at constant temperature and pressure with gas burette. A few investigators have used the Barcroft-Warburg apparatus in which the differences in pressure as the reaction proceeds are read on a manometer while the volume and temperature are kept constant (5, 8).

For the present investigation the latter technique had real advantages. It was possible to obtain information rapidly on a large number of small samples, thereby avoiding useless waste of valuable material, and the samples were large enough to use for the subsequent chemical and polarographic analyses.

Experimental

Materials Used. The methyl oleate (A) whose oxidation is reported in this paper was prepared from olive oil by low temperature crystallization and distil-

lation (7), except that the free acid was crystallized three times at -55° before esterification. This material had an iodine number of 84.9, and a composition of 99.0% methyl oleate, 0.9% saturates, and 0.07% methyl linoleate (ultraviolet absorption method).

Mention will also be made of the oxidation of eight samples from a second, but slightly less pure, methyl oleate (B) (10). This ester closely approximated the first in iodine value and content of methyl oleate and saturated esters, but it had a methyl linoleate content of 0.2%.

Measurement of Oxygen Absorption. The Barcroft-Warburg apparatus and the methods used to measure oxygen absorption were similar to those described by Johnston and Frey (5) with the following exceptions. The reaction flasks contained no inner cup, mercury was used as a manometer fluid (8), and the step in the cleaning procedure involving the use of cleaning solution was replaced by heating all glassware coming in contact with the sample in concentrated sulfuric-nitric acid solution for two hours at 150° , followed by rinsing in distilled water, boiling in 0.001 N sodium hydroxide, and rinsing again in distilled water before proceeding with steaming and drying.

Preliminary experiments showed that when the thickness of a sample film was less than 0.5 mm., variations in surface area and agitation had little effect on the rate of oxidation. The use of a 1-cc. sample yielded a film with an average thickness of 0.4 mm.

A series of six (occasionally five) 1-cc. samples were oxidized at one time. The oxidation of the samples was terminated at different predetermined points to obtain a wide distribution in the extent of oxidation. The oxidation of subsequent series at the same temperature was terminated to overlap and fall between values for a previous series of samples.

Determination of Peroxides. A modification of the method of Wheeler (11) was used for the chemical determination of total peroxides. An aliquot of 40-120 mg. of the oxidized methyl oleate sample dissolved in 10 cc. of the glacial acetic acid-chloroform solution was treated with 1 cc. of freshly prepared 50% potassium iodide solution for 15 min. under nitrogen followed by dilution with 25 cc. of distilled water and titration with 0.01 N thiosulfate solution.

The polarographic determination of hydroperoxide has been described previously (12).

Total oxygen absorbed, chemically determined peroxides, and polarographically determined hydroperoxides have all been expressed as millimoles of oxygen per mole of original methyl oleate.

Results and Discussion

Autoxidation of Methyl Oleate. Samples of specially purified methyl oleate (A) were oxidized at 100, 80, and 60° (Figure 1). In the early stages of autoxida-

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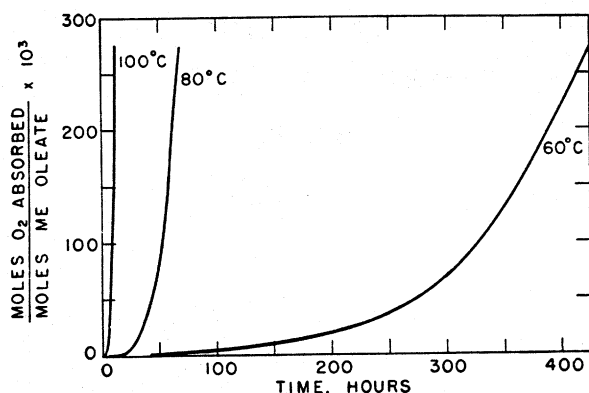


FIG. 1. Millimoles of oxygen absorbed per mole of methyl oleate against time in hours.

tion the rate of oxygen absorption varied from sample to sample at a given temperature and level of oxidation. As the period of rapid acceleration of the reaction was passed however, it became approximately the same for all samples at a given temperature.

A plot of the oxygen uptake against time resulted in a family of curves for samples autoxidized at each temperature. These curves diverged at first but became substantially parallel as the acceleration of the oxidation rate decreased. This parallelism in the rate of oxidation at a given level of oxidation and temperature was evident in a majority of samples by the time 80 millimoles of oxygen had been absorbed, and in nearly all samples when 100 millimoles of oxygen had reacted. Comparison of the median curves selected from the three families obtained at 100, 80, and 60° (Figure 1), at the point where 100 millimoles of oxygen had been absorbed per mole of methyl oleate, showed the rates of oxidation were approximately 45, 7, and 1.25 millimoles of oxygen absorbed per mole of methyl oleate per hour, respectively, or a ratio of 36:5.6:1.

Relation of Peroxide Content to Oxygen Absorbed. The polarographic method for determining hydroperoxides in the presence of other autoxidation products is specific, with the exception of aliphatic 1,2-diketones whose half-wave potentials overlap. The presence of this latter class of compound is unlikely in the early stages of autoxidation of methyl oleate. The iodometric (chemical) method determines hydroperoxides and other peroxide types, with the exception of dialkyl peroxides, whose presence in significant amounts is unlikely.

In the oxidations described in this paper 1.6-30.6% of the methyl oleate was oxidized, assuming one mole

of oxygen absorbed to one mole of methyl oleate. Twenty-two samples were studied at 100°, 15 at 80°, and 21 at 60°. All of the voluminous data obtained are not reported here. Seven representative samples, more or less regularly spaced over the reported range of oxidation, were selected from the group oxidized at each temperature. The results are summarized in Table I.

The following conclusions, derivable from Table I, are noteworthy. The ratio of chemical peroxide to total oxygen absorbed (columns 1, 4, and 7, Table I) is substantially the same at a given oxidation level, regardless of the temperature, with the ratio decreasing as oxidation proceeds. Even in the earliest stages, when less than 2% of the methyl oleate has been oxidized, about 10% of the oxygen absorbed is present in groups other than peroxide. The amount of non-peroxidic oxygen, as would be anticipated, increases as the oxidation proceeds, until at about 300 millimoles of oxygen absorbed per mole of methyl oleate, about 30% of the oxygen is in non-peroxidic groups. This increase in non-peroxidic oxygen is undoubtedly a result largely of peroxide decomposition to other species.

The ratios of hydroperoxide to total oxygen absorbed at 100° and 80° (columns 2 and 5, Table I) parallel the peroxide-oxygen ratios but are slightly lower over the entire oxidation range. These results confirm the conclusions of earlier workers that hydroperoxide formation is by far the major reaction occurring.

At 60°, and only at this temperature, there is poor correlation among the four replicate series of oxidations in the percentage of absorbed oxygen accounted for as hydroperoxide (column 8, Table I). Our explanation for this is that the protracted period of autoxidation at this low temperature, involving at least 48 hrs. before any measurable oxygen uptake occurred and an additional 400 hrs. before 300 millimoles had been absorbed, allows slight variations in experimental conditions to influence secondary reactions, giving rise to variable results among replicates. At higher temperatures however rates of primary reactions are sufficiently rapid to overshadow such minor variations.

The standard deviation of the difference between duplicates for the chemical (iodometric) and polarographic methods have been calculated. Standard deviations of 0.080, 0.064, and 0.131% were obtained for the chemical method at 100°, 80°, and 60°, respectively, and 0.229, 0.141, and 0.112% for the polarographic method.

TABLE I
Relationship of Chemical Peroxide, Hydroperoxide, and Total Oxygen Absorbed in the Autoxidation of Specially Purified Methyl Oleate (A)

100°			80°			60°		
Chemical peroxide, % ^a	Hydroperoxide, % ^a	Ratio hydroperoxide to chemical peroxide, %	Chemical peroxide, % ^a	Hydroperoxide, % ^a	Ratio hydroperoxide to chemical peroxide, %	Chemical peroxide, % ^a	Hydroperoxide, % ^a	Ratio hydroperoxide to chemical peroxide, %
91 (16) ^b	89	98	92 (17) ^b	80	88	89 (16) ^b	91	102
82 (53)	84	102	86 (57)	78	91	88 (62)	75	85
82 (98)	77	94	83 (97)	79	95	81 (101)	85	105
79 (153)	73	92	78 (173)	72	92	83 (147)	80	96
77 (206)	70	91	77 (214)	73	94	79 (200)	71	90
72 (254)	74	103	75 (260)	71	94	77 (236)	72	94
71 (303)	67	94	72 (306)	66	93	74 (298)	81	110

^a These percentages are the ratio of the chemical peroxide or hydroperoxide content to total oxygen absorbed.

^b The values in parentheses are total oxygen absorbed in millimoles per mole of methyl oleate. The figures are given to show the overall extent of oxidation.

Figures 2, 3, and 4 are plots of peroxide and hydroperoxide content against oxygen absorbed by samples at 100, 80, and 60°. In the interest of clarity only a portion of the values, selected at random, are shown, but all values between 16 and 306 millimoles of oxygen have been used to calculate the equations to be discussed shortly. All values are expressed as millimoles of oxygen per mole of original methyl oleate. Values of the chemical peroxide and hydroperoxide are plotted on the y-axis and total oxygen absorbed on the x-axis.

The plots are almost, but not quite, straight lines. Actually a plot of the logarithms of these values did give straight lines and, by the method of least squares, we were able to obtain the equations which fit these values. Table II summarizes the equations for the

TABLE II
Equations Relating Peroxide^a to Total Oxygen Uptake

t°C.	Chemical peroxide	Hydroperoxide
100	$y_c = 1.14 x^{0.925}$	$y_h = 1.07 x^{0.928}$
80	$y_c = 1.16 x^{0.924}$	$y_h = 0.98 x^{0.913}$
60	$y_c = 1.00 x^{0.958}$	$y_h = 0.94 x^{0.964}$
60-100	$y_c = 1.09 x^{0.936}$	$y_h = 1.02 x^{0.936}$

^a y_c = chemical peroxide; y_h = hydroperoxide; x = oxygen absorbed.

variation of peroxide and hydroperoxide content with oxygen absorbed at the three temperatures investigated. Since the equations are similar for 100, 80, and 60°, a generalized equation for chemically determined peroxide (y_c) fitting the data in the range 0-100° has been obtained (Table II). Similarly, a generalized equation for the 80-100° range (omitting the results at 60°) has been obtained for the hydroperoxide content (y_h). The values for standard deviations mentioned previously show that these parabolic equations are a better fit for the six sets of data than straight lines since, for a given set of data, the deviations of the points from the best straight line that can be drawn through the points are much greater than can be accounted for by the standard error of the methods. These deviations become more pronounced as the autoxidation proceeds, as can be seen

Figures 2, 3, and 4. The curves calculated from the derived equations of Table II fit the data well up to an oxidation level of 200 millimoles of absorbed oxygen for chemical peroxide at all three temperatures and for hydroperoxide at 100 and 80°. The fact that at above 200 millimoles of absorbed oxygen these equations predict too high values emphasizes once again that as the oxidation becomes more complex, simple generalizations become less applicable.

The equation derived from the hydroperoxide data at 60° corresponds closely to the other equations in Table II. It is not of much value however in predicting hydroperoxide values since, as mentioned earlier, this temperature replicate series of oxidations do not agree well in the absorbed oxygen accounted for by hydroperoxide.

The curve derived from the generalized equation for the variation of the chemically determined peroxide content with oxygen uptake in the range 60-100° is shown in Figure 5. For clarity, about 24 points out of 58 are omitted at random from the plot.

In the early stages of oxidation the values for all three temperatures are fitted by this curve admirably. When about 150 millimoles of oxygen has been ab-

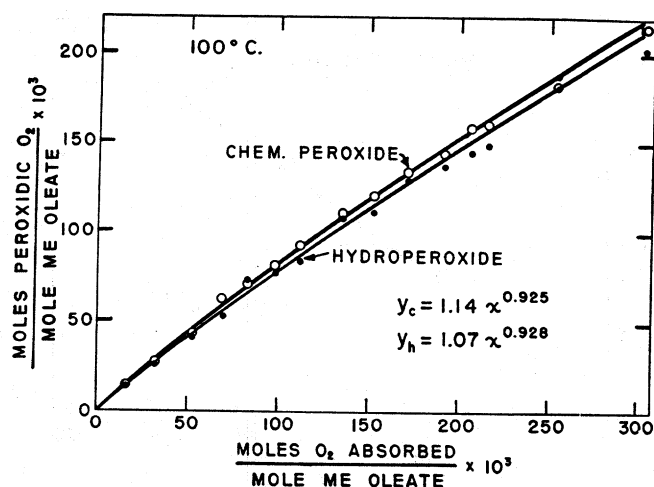


Fig. 2. Millimoles of peroxidic oxygen (chemically determined and hydroperoxide) per mole of methyl oleate against millimoles of oxygen absorbed per mole of methyl oleate at 100°.

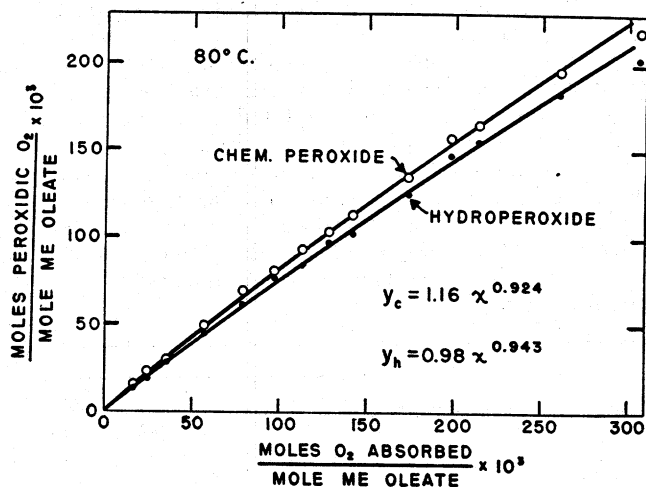


Fig. 3. Millimoles of peroxidic oxygen (chemically determined and hydroperoxide) per mole of methyl oleate against millimoles of oxygen absorbed per mole of methyl oleate at 80°.

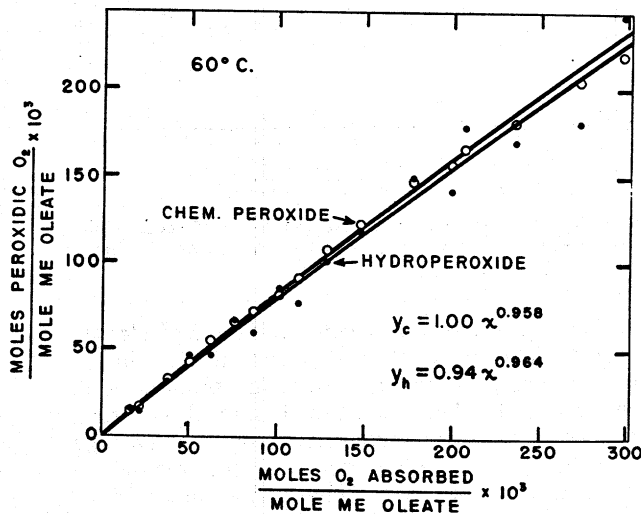


Fig. 4. Millimoles of peroxidic oxygen (chemically determined and hydroperoxide) per mole of methyl oleate against millimoles of oxygen absorbed per mole of methyl oleate at 60°.

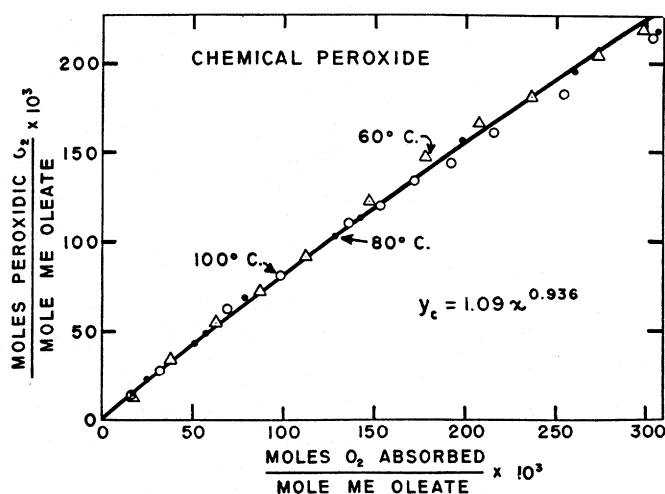


Fig. 5. Millimoles of chemically determined peroxide per mole of methyl oleate against millimoles of oxygen absorbed per mole of methyl oleate at 60-100°.

sorbed however, the curve predicts too high a peroxide content for oxidations at 100° and, for a time, too low for oxidations at 60°. At this point more complex oxidative reactions are setting in, which are apparently affected differently by temperature.

Figure 6 shows the curve calculated from the generalized equation derived from the hydroperoxide data at 100 and 80° only. Again up to the point where 150 millimoles of oxygen have been absorbed per mole of methyl oleate, the data fit the curve well, with little deviation. Beyond this value the deviation above or below the curve is greater but is random for both temperatures.

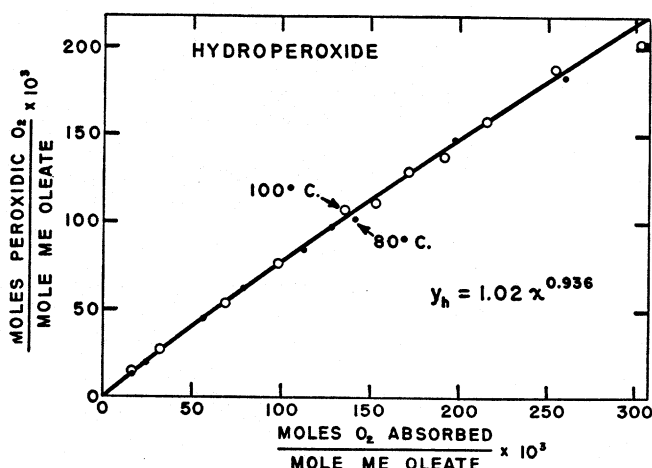


Fig. 6. Millimoles of hydroperoxide per mole of methyl oleate against millimoles of oxygen absorbed per mole of methyl oleate at 80 and 100°.

The Relation of Hydroperoxide to Chemical Peroxide Content. The ratio of hydroperoxide to chemically determined peroxide is shown in columns 3, 6, and 9 of Table I. This ratio, although fluctuating somewhat from sample to sample, is on the average fairly constant and independent of the extent to which a sample is oxidized. The ratios obtained at 80° are the most consistent at all levels of oxidation, a condition which is true of all other data obtained from the samples oxidized at 80°. We attribute this

fact to less variability in oxidative reactions at this temperature.

Table III summarizes the ratio of hydroperoxide to chemical peroxide for the specially purified (A) and the slightly less pure (B) methyl oleate. The values in the second column are derived from data discussed in the previous part of this paper and obtained from the oxidation at 100, 80, and 60° of the more highly purified methyl oleate (A) having an induction period of 12 hours at 80° and containing 0.07% methyl linoleate. Most but not all of the total peroxides are accounted for as hydroperoxide, the least amount (93%) being found at 80°. The most consistent values for hydroperoxide and peroxide content are also obtained at 80°, lending increased validity to the above value.

TABLE III
Ratio (%) of Hydroperoxide to Chemical Peroxide

t°C.	Methyl oleate (A)	Methyl oleate (B)
100	95.9	
80	93.0	85.4
60	97.3	

The third column shows the average of results obtained from the oxidation at 80° of eight samples of the less pure methyl oleate (B) having an induction period of 5 hrs. and containing 0.2% methyl linoleate. The difference between the total peroxides and hydroperoxide is 14.6% while that for the more highly purified methyl oleate preparation at the same temperature is only 7%. Samples of these two preparations differed primarily in methyl linoleate content but were handled in the same manner.

The nature and manner of formation of the non-hydroperoxide portion of methyl oleate peroxides is still not known. Swern and co-workers (9) have suggested that the non-hydroperoxide portion is probably cyclic, at least in part. Several of their oxidized methyl oleate samples contained significant proportions of non-hydroperoxides among the total peroxidic compounds. In one sample oxidized at 100° in the presence of ultraviolet to a peroxide content of 28.6%, as much as 28% of the total peroxide was not hydroperoxide.

The question of why the proportion of non-hydroperoxides among the total peroxidic compounds varies for different autoxidized methyl oleate preparations still remains unanswered. Comparison of the results obtained by us with the two methyl oleate preparations (A) and (B) suggests the possibility that the same impurities which shorten the induction period and accelerate the autoxidation also contribute to the complexity of the total peroxides formed. Further investigation is indicated.

Summary

Methyl oleate has been autoxidized at 100°, 80°, and 60° in the Barcroft-Warburg apparatus. Samples have been analyzed for total peroxide by the iodometric method and for hydroperoxide by the polarographic method. These peroxide values have been compared with each other and with total oxygen absorbed.

The relation of chemical peroxide (y_c) to oxygen uptake (x) is expressed by $y_c = 1.09x^{0.936}$. This equa-

tion is equally valid at the three temperatures for the first 150 millimoles (15%) of oxygen absorbed per mole of methyl oleate.

Similarly, the hydroperoxide content (y_h) for the first 150 millimoles of oxygen absorbed at 80° and 100° is given by the equation $y_h = 1.02x^{0.936}$.

The ratio of hydroperoxide to chemically determined peroxide was, on the whole, constant throughout the entire range of oxidation (15-300 millimoles of oxygen absorbed per mole), and averaged about 95%.

It has been shown unequivocally that the major portion of the peroxides formed in the autoxidation of methyl oleate are hydroperoxides, confirming conclusions of recent investigators. A small but significant amount of non-hydroperoxidic peroxide appears to be formed concurrently.

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